

WHAT IS CLAIMED IS:

- 1                   1.       An isolated protein comprising a HER-2/neu extracellular domain  
2       fused to a HER-2/neu phosphorylation domain, wherein the protein is capable of  
3       producing an immune response in a warm-blooded animal.
- 1                   2.       The protein of claim 1, wherein the protein has a sequence at least  
2       80% identical to the sequence of SEQ ID NO:6, or wherein the protein comprises a  
3       sequence at least 80% identical to the sequence of SEQ ID NO:3 fused to a sequence at  
4       least 80% identical to the sequence of SEQ ID NO:4.
- 1                   3.       The protein of claim 1, wherein the protein comprises a sequence at  
2       least 80 % identical to the sequence of SEQ ID NO:3 directly fused to an amino acid  
3       sequence at least 80% identical to the sequence inclusive of Gln 991 to Val 1256 of SEQ  
4       ID NO:2, or wherein the protein comprises a sequence at least 80 % identical to the  
5       sequence of SEQ ID NO:3 fused to the amino acid sequence at least 80% identical to the  
6       sequence inclusive of Gln 991 to Val 1256 of SEQ ID NO:2.
- 1                   4.       The protein of claim 1, wherein the protein comprises a sequence at  
2       least 80% identical to the sequence of SEQ ID NO:8 directly fused to a sequence at least  
3       80% identical to the sequence of SEQ ID NO:4, or wherein the protein comprises a  
4       sequence at least 80% identical to the sequence of SEQ ID NO:8 fused to a sequence at  
5       least 80% identical to the sequence of SEQ ID NO:4.
- 1                   5.       The protein of claim 1, wherein the protein comprises a sequence at  
2       least 80% identical to the sequence of SEQ ID NO:8 directly fused to the amino acid  
3       sequence inclusive of Gln 991 to Val 1256 of SEQ ID NO:2, or wherein the protein  
4       comprises a sequence at least 80% identical to the sequence of SEQ ID NO:8 fused to a  
5       sequence at least 80% identical to the amino acid sequence inclusive of Gln 991 to Val  
6       1256 of SEQ ID NO:2.
- 1                   6.       The protein of claim 1, wherein the HER-2/neu extracellular  
2       domain is fused to the HER-2/neu phosphorylation domain via a chemical linker.
- 1                   7.       The protein of claim 6, wherein the chemical linker is an amino  
2       acid linker.



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- 1                   8.     A nucleic acid molecule encoding the protein of claim 1.
- 1                   9.     A viral vector comprising a polynucleotide sequence encoding the  
2 protein of claim 1.
- 1                   10.    A pharmaceutical composition comprising the protein of claim 1,  
2 and a pharmaceutically acceptable carrier or diluent.
- 1                   11.    The pharmaceutical composition of claim 10, wherein the  
2 pharmaceutical composition is a vaccine.
- 1                   12.    The pharmaceutical composition of claim 10, further comprising an  
2 immunostimulatory substance.
- 1                   13.    The pharmaceutical composition of claim 12, wherein the protein is  
2 presented in an oil-in-water emulsion.
- 1                   14.    The pharmaceutical composition of claim 12, wherein the  
2 immunostimulatory substance is SBAS2, 3D-MPL, QS21, or a combination of 3D-MPL  
3 and QS21.
- 1                   15.    A pharmaceutical composition comprising the nucleic acid  
2 molecule of claim 8, and a pharmaceutically acceptable carrier or diluent.
- 1                   16.    The pharmaceutical composition of claim 15, wherein the  
2 pharmaceutical composition is a vaccine.
- 1                   17.    The pharmaceutical composition of claim 15, further comprising an  
2 immunostimulatory substance.
- 1                   18.    The pharmaceutical composition of claim 15, wherein the nucleic  
2 acid molecule is a DNA molecule.
- 1                   19.    A method for eliciting or enhancing an immune response to HER-  
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal  
3 the protein of claim 1 in an amount effective to elicit or enhance the immune response.



1                   20.     The method of claim 19, wherein the protein is administered in the  
2     form of a vaccine.

1                   21.     A method for eliciting or enhancing an immune response to HER-  
2     2/neu protein, the method comprising the step of administering to a warm-blooded animal  
3     the nucleic acid molecule of claim 8 in an amount effective to elicit or enhance the  
4     immune response.

1                   22.     The method of claim 21, wherein the nucleic acid molecule is in  
2     the form of a vaccine.

1                   23.     The method of claim 21, wherein the step of administering  
2     comprises transfecting cells of the warm-blooded animal *ex vivo* with the nucleic acid  
3     molecule and subsequently delivering the transfected cells to the warm-blooded animal.

1                   24.     A method for eliciting or enhancing an immune response to HER-  
2     2/neu protein, the method comprising the step of administering to a warm-blooded animal  
3     the viral vector of claim 9 in an amount effective to elicit or enhance the immune  
4     response.

1                   25.     The method of claim 24, wherein the step of administering  
2     comprises infecting cells of the warm-blooded animal *ex vivo* with the viral vector and  
3     subsequently delivering the infected cells to the warm-blooded animal.

1                   26.     An isolated protein comprising a HER-2/neu extracellular domain  
2     fused to a fragment of the HER-2/neu phosphorylation domain, wherein the protein is  
3     capable of producing an immune response in a warm-blooded animal.

1                   27.     The protein of claim 26, wherein the protein has a sequence at least  
2     80% identical to the sequence of SEQ ID NO:7, or wherein the protein comprises a  
3     sequence at least 80% identical to the sequence of SEQ ID NO:3 fused to a sequence at  
4     least 80% identical to the sequence of SEQ ID NO:5.

1                   28.     The protein of claim 26, wherein the protein comprises a sequence  
2     at least 80% identical to the sequence of SEQ ID NO:3 directly fused to a sequence at  
3     least 80% identical to the amino acid sequence inclusive of Gln 991 to Arg 1049 of SEQ



4 ID NO:2, or wherein the protein comprises a sequence at least 80% identical to the  
5 sequence of SEQ ID NO:3 fused to a sequence at least 80% identical to the amino acid  
6 sequence inclusive of Gln 991 to Arg 1049 of SEQ ID NO:2.

1 29. The protein of claim 26, wherein the protein comprises a sequence  
2 at least 80% identical to the sequence of SEQ ID NO:8 directly fused to a sequence at  
3 least 80% identical to the sequence of SEQ ID NO:5, or wherein the protein comprises a  
4 sequence at least 80% identical to the sequence of SEQ ID NO:8 fused to a sequence at  
5 least 80% identical to the sequence of SEQ ID NO:5.

1 30. The protein of claim 26, wherein the protein comprises a sequence  
2 at least 80% identical to the sequence of SEQ ID NO:8 directly fused to a sequence at  
3 least 80% identical to the amino acid sequence inclusive of Gln 991 to Arg 1049 of SEQ  
4 ID NO:2, or wherein the protein comprises a sequence at least 80% identical to the  
5 sequence of SEQ ID NO:8 fused to a sequence at least 80% identical to the amino acid  
6 sequence inclusive of Gln 991 to Arg 1049 of SEQ ID NO:2.

1 31. The protein of claim 26, wherein the HER-2/neu extracellular  
2 domain is fused to the fragment of the HER-2/neu phosphorylation domain via a chemical  
3 linker.

1 32. The protein of claim 31, wherein the chemical linker is an amino  
2 acid linker.

1 33. A nucleic acid molecule encoding the protein of claim 26.

1 34. A viral vector comprising a polynucleotide sequence encoding the  
2 protein of claim 26.

1 35. A pharmaceutical composition comprising the protein of claim 26,  
2 and a pharmaceutically acceptable carrier or diluent.

1 36. The pharmaceutical composition of claim 35, wherein the  
2 pharmaceutical composition is a vaccine.

1 37. The pharmaceutical composition of claim 35, further comprising an  
2 immunostimulatory substance.







49. A method for eliciting or enhancing an immune response to HER-2/neu protein, the method comprising the step of administering to a warm-blooded animal the viral vector of claim 34 in an amount effective to elicit or enhance the immune response.

50. The method of claim 49, wherein the step of administering comprises infecting cells of the warm-blooded animal *ex vivo* with the viral vector and subsequently delivering the infected cells to the warm-blooded animal.

51. An isolated protein comprising a HER-2/neu extracellular domain fused to a HER-2/neu intracellular domain, wherein the protein is capable of producing an immune response in a warm-blooded animal.

52. The protein of claim 51, wherein the protein comprises a sequence at least 80% identical to the sequence of SEQ ID NO:3 fused directly to a sequence at least 80% identical to the amino acid sequence inclusive of Lys 676 to Val 1255 in SEQ ID NO:1, or wherein the protein comprises a sequence at least 80% identical to the sequence of SEQ ID NO:3 fused to a sequence at least 80% identical to the amino acid sequence inclusive of Lys 676 to Val 1255 of SEQ ID NO:1 via at least one of a chemical or amino acid linking group.

53. The protein of claim 51, wherein the protein comprises a sequence at least 80% identical to the sequence of SEQ ID NO:3 directly fused to a sequence at least 80% identical to the amino acid sequence inclusive of Lys 677 to Val 1256 of SEQ ID NO:2, or wherein the protein comprises a sequence at least 80% identical to the sequence of SEQ ID NO:3 fused to a sequence at least 80% identical to the amino acid sequence inclusive of Lys 677 to Val 1256 of SEQ ID NO:2 via at least one of a chemical or amino acid linking group.

54. The protein of claim 51, wherein the protein comprises a sequence at least 80% identical to the sequence of SEQ ID NO:8 directly fused to a sequence at least 80% identical to the amino acid sequence inclusive of Lys 676 to Val 1255 of SEQ ID NO:1, or wherein the protein comprises a sequence at least 80% identical to the sequence of SEQ ID NO:8 fused to a sequence at least 80% identical to the amino acid



6 sequence inclusive of Lys 676 to Val 1255 of SEQ ID NO:1 via at least one of a chemical  
7 or amino acid linking group.

1                    55.        The protein of claim 51, wherein the protein comprises a sequence  
2        at least 80% identical to the sequence of SEQ ID NO:8 directly fused to a sequence at  
3        least 80% identical to the amino acid sequence inclusive of Lys 677 to Val 1256 of SEQ  
4        ID NO:2, or wherein the protein comprises a sequence at least 80% identical to the  
5        sequence of SEQ ID NO:8 fused to a sequence at least 80% identical to the amino acid  
6        sequence inclusive of Lys 677 to Val 1256 of SEQ ID NO:2 via at least one of a chemical  
7        or amino acid linking group.

1                    56.     The protein of claim 51, wherein the HER-2/neu extracellular  
2     domain is fused to the HER-2/neu intracellular domain via a chemical linker.

1                    57.        The protein of claim 56, wherein the chemical linker is an amino  
2        acid linker.

1            58.    A nucleic acid molecule encoding the protein of claim 51.

1                    59.        A viral vector comprising a polynucleotide sequence encoding the  
2        protein of claim 51.

1                    60.     A pharmaceutical composition comprising the protein of claim 51,  
2     and a pharmaceutically acceptable carrier or diluent.

1            61.    The pharmaceutical composition of claim 60, wherein the  
2    pharmaceutical composition is a vaccine.

1                   62.     The pharmaceutical composition of claim 60, further comprising an  
2     immunostimulatory substance.

1                    63.     The pharmaceutical composition of claim 62, wherein the protein is  
2     presented in an oil-in-water emulsion.

1                    64.        The pharmaceutical composition of claim 62, wherein the  
2 immunostimulatory substance is SBAS2, 3D-MPL, QS21, or a combination of 3D-MPL  
3 and QS21.



1                   65.    A pharmaceutical composition comprising the nucleic acid  
2 molecule of claim 58, and a pharmaceutically acceptable carrier or diluent.

1                   66.    The pharmaceutical composition of claim 65, wherein the  
2 pharmaceutical composition is a vaccine.

1                   67.    The pharmaceutical composition of claim 65, further comprising an  
2 immunostimulatory substance.

1                   68.    The pharmaceutical composition of claim 65, wherein the nucleic  
2 acid molecule is a DNA molecule.

1                   69.    A method for eliciting or enhancing an immune response to HER-  
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal  
3 the protein of claim 51 in an amount effective to elicit or enhance the immune response.

1                   70.    The method of claim 69, wherein the protein is administered in the  
2 form of a vaccine.

1                   71.    A method for eliciting or enhancing an immune response to HER-  
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal  
3 the nucleic acid molecule of claim 58 in an amount effective to elicit or enhance the  
4 immune response.

1                   72.    The method of claim 71, wherein the nucleic acid molecule is in  
2 the form of a vaccine.

1                   73.    The method of claim 71, wherein the step of administering  
2 comprises transfecting cells of the warm-blooded animal *ex vivo* with the nucleic acid  
3 molecule and subsequently delivering the transfected cells to the warm-blooded animal.

1                   74.    A method for eliciting or enhancing an immune response to HER-  
2 2/neu protein, the method comprising the step of administering to a warm-blooded animal  
3 the viral vector of claim 59 in an amount effective to elicit or enhance the immune  
4 response.



1                   75.     The method of claim 74, wherein the step of administering  
2     comprises infecting cells of the warm-blooded animal *ex vivo* with the viral vector and  
3     subsequently delivering the infected cells to the warm-blooded animal.

1                   76.     A method for inhibiting the development of a cancer in a patient,  
2     the method comprising the step of administering to a patient an effective amount of a  
3     fusion polypeptide according to claim 1, 26, or 51 and thereby inhibiting the development  
4     of a cancer in the patient.

1                   77.     A method for inhibiting the development of a cancer in a patient,  
2     the method comprising the step of administering to a patient an effective amount of a  
3     polynucleotide according to claim 8, 33, or 58 and thereby inhibiting the development of  
4     a cancer in the patient.

1                   78.     A method for inhibiting the development of a cancer in a patient,  
2     the method comprising the step of administering to a patient an effective amount of an  
3     antigen-presenting cell that expresses a fusion polypeptide according to claim 1, 26, or  
4     51, and thereby inhibiting the development of a cancer in the patient.

1                   79.     A method according to claim 78, wherein the antigen-presenting  
2     cell is a dendritic cell.

1                   80.     A method according to any one of claims 76-79, wherein the  
2     cancer is breast, ovarian, colon, lung or prostate cancer.

1                   81.     A method for removing tumor cells from a biological sample, the  
2     method comprising the step of contacting a biological sample with T cells that  
3     specifically react with a HER-2/neu fusion protein, wherein the fusion protein comprises  
4     an amino acid sequence that is encoded by a polynucleotide sequence selected from the  
5     group consisting of:

6                             (i)     polynucleotides recited in any one of SEQ ID NO:8, 33, or  
7     58; and

8                             (ii)    complements of the foregoing polynucleotides;

9                             wherein the step of contacting is performed under conditions and for a  
10    time sufficient to permit the removal of cells expressing the antigen from the sample.



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1                   82.     A method according to claim 81, wherein the biological sample is  
2 blood or a fraction thereof.

1                   83.     A method for inhibiting the development of a cancer in a patient,  
2 comprising the step of administering to a patient a biological sample treated according to  
3 the method of claim 81.

1                   84.     A method for stimulating and/or expanding T cells specific for a  
2 HER-2/neu fusion protein, the method comprising the step of contacting T cells with one  
3 or more of:

- 4                   (i)     a fusion protein according to claims 1, 26, or 51;  
5                   (ii)    a polynucleotide encoding such a fusion protein; or  
6                   (iii)   an antigen presenting cell that expresses such a fusion protein;  
7                   under conditions and for a time sufficient to permit the stimulation and/or  
8 expansion of T cells.

1                   85.     An isolated T cell population, comprising T cells prepared  
2 according to the method of claim 84.

1                   86.     A method for inhibiting the development of a cancer in a patient,  
2 the method comprising the step of administering to a patient an effective amount of a T  
3 cell population according to claim 85.

1                   87.     A method for inhibiting the development of a cancer in a patient,  
2 the method comprising the steps of:

- 3                   (a)     incubating CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells isolated from a patient with  
4 at least one component selected from the group consisting of:

- 5                   (i)     a fusion protein according to claims 1, 26, or 51;  
6                   (ii)    a polynucleotide encoding such a fusion protein; and  
7                   (iii)   an antigen-presenting cell that expresses such a fusion  
8 protein;

9                   such that T cells proliferate; and

- 10                  (b)     administering to the patient an effective amount of the proliferated  
11 T cells, thereby inhibiting the development of a cancer in the patient.



1                   88.    A method for inhibiting the development of a cancer in a patient,  
2   the method comprising the steps of:

3                   (a)    incubating CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells isolated from a patient with  
4   at least one component selected from the group consisting of:

5                           (i)    a fusion protein according to claims 1, 26, or 51;  
6                           (ii)   a polynucleotide encoding such a fusion protein; and  
7                           (iii)  an antigen-presenting cell that expresses such a fusion  
8   protein;

9                   such that T cells proliferate;

10                  (b)    cloning at least one proliferated cell; and

11                  (c)    administering to the patient an effective amount of the cloned T  
12   cells, thereby inhibiting the development of a cancer in the patient.

1                   89.    A method of making a fusion protein according to claims 1, 26, or  
2   51, the method comprising the steps of:

3                   (a)    introducing into a cell an expression vector comprising a  
4   polynucleotide according to claims 8, 33, or 58;

5                           (b)   culturing the transfected cell; and

6                           (c)   purifying the expressed protein.

1                   90.    The method of claim 89, wherein the cell is a CHO cell.

1                   91.    The method of claim 89, wherein the cell is cultured in suspension,  
2   under serum-free conditions.

1                   92.    The method of claim 89, wherein the expressed protein is purified  
2   by a two-step procedure, the procedure comprising:

3                   (a)    anion exchange chromatography on Q sepharose High Performance  
4   Columns; and

5                   (b)    hydrophobic chromatography on Phenyl Sepharose 6 Fast Flow  
6   low substitution.